

## Physico-chemical, Sensory and Microbial Analysis of Wine Produced from Watermelon (*Citrullus Lanatus*) and Pawpaw (*Carica Papaya*) Blend.

\*<sup>1</sup>Adedeji, T.O. and <sup>2</sup>Oluwalana, I.B.

*E-mail of the corresponding author:* [temileyeadejideji@yahoo.com](mailto:temileyeadejideji@yahoo.com), Tel: +234 8066514332

<sup>1</sup>Dept of Food Science and Technology, Osun State Polytechnic, P.M.B. 301, Iree, Nigeria

<sup>2</sup>Dept. of Food Science and Technology, Federal University of Technology, P.M.B. 704, Akure, Nigeria.

### Abstract

The study was conducted to develop a ready-to-serve (RTS) wine using pawpaw (*Carica papaya*) and watermelon (*Citrullus lanatus*) blend using the following ratio PJ:WMJ, 90:10, 80:20, 70:30: 60:40 50:50 with sugars, distilled water, and potassium metabisulphite and yeast (*Saccharomyces cerevisiae*). The results of physico-chemical analysis revealed that titratable acidity ranged from 1.00 to 1.70% lactic acid while pH from 2.00-3.00, total soluble solids (brix level) ranged between 12.00-13.00<sup>0</sup>brix, total solid varied significantly from 1.50-2.00%, and specific gravity from 0.784 - 1.020%. Vitamin C contents ranged from 7.00-9.00x10<sup>-4</sup>mg/100g while vitamin B1 content ranged from 8.80 to 10.00mg/100g and Vitamin A ranged from 20.00-24.00mg/100g. Samples subjected to sensory evaluation revealed that there were no significant difference at (p < 0.05) among the wine samples with respect to colour, aroma, taste, consistence, and overall acceptability. Findings of microbial studies showed tolerable no of total plate counts in the formulated wine which is safe for human consumption. From the result of quality assessments, the formulated wine sample coded 672 was found to be superior in quality and could be stored at 30 ± 2<sup>0</sup>C for a minimum period of six months without any significant changes in quality.

**Keywords:** watermelon, pawpaw, physicochemical, microbiological, sensory

### 1. Introduction

Watermelon (*Citrullus lanatus*) is a fruit which belongs to the family of cucurbitacea. The fruit is round with reddish mesocarp having a lot of seed and is mostly common in the south. There are various species with different coloured endocarp for example, red flesh, yellow flesh and orange flesh. It contains vitamin B1 and B6, potassium and magnesium in addition to vitamin A and C which is generally common to all fruits and vegetables (Abdelwahab *et. al.*, 2011). Watermelon (*Citrullus lanatus*) is rich in carotenoids some of which include lycopene, phytofluene, phytoene, beta-carotene, lutein and neurospene. Lycopene makes up the majority of the carotenoids of watermelon. Carotenoids have antioxidant activity and free scavenging property thereby help in reducing the risk of cancers, cardiovascular diseases, arteriosclerosis diabetes and arthritis and protects against macular degeneration. A watermelon is nominally 60% flesh and about 90% of the flesh is juicy which contains 7 to 10% (w/v) sugar. Thus, over 50% of the watermelon is readily fermentable liquid (Altas, *et. al.*, 2011)

Watermelon is an unusual fruit source of the carotenoid lycopene and a rich source of phenolic antioxidants. Watermelon contains cucurbitacin E, a triterpene anti-inflammatory phytonutrient, and unusual amounts of the amino acid citrulline. Watermelon is an excellent source of immune-supportive vitamin C. It is also a very good source of free-radical-scavenging vitamin A (in several carotenoid forms, and especially in the specific form of beta-carotene) (Dimitrovski *et.al.*, 2010). In addition, watermelon is a good source of heart-healthy potassium and magnesium. The nutritional profile of watermelon is full array of nutrients, including carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids and more. A serving cup of watermelon contains 12.31mg of vitamin C, 864.88IU of vitamin A 170.24MG of potassium and 45.60 calories. The nutritional profile of watermelon is full array of nutrients, including carbohydrates, sugar, soluble and insoluble fiber, sodium, vitamins, minerals, fatty acids, amino acids and more. A serving cup of watermelon contains 12.31mg of vitamin C, 864.88IU of vitamin A 170.24MG of potassium and 45.60 calories (Charoensiri *et al.*, 2009)

Pawpaw (*Carica papaya*) is a fruit which belongs to the family of annonace. It is usually oblong in shape and 5 – 15cm in length (Svans, 2008). There are more than 40 varieties of pawpaw with different coloured endocarp for

example, red flesh, yellow flesh and orange flesh. It grows in temperate climate, pleasant in taste and has great application in the production of food confectioneries such as jam and can be used to treat worms. It also contains protein (0.6%), moisture (85%) and sugar (10-13%). It is very rich in vitamin A and C (Anon, 2008). The fruit's high nutritional quality also makes it an excellent contribution to a balanced diet. It is also a potential source of natural fruit flavor. Pawpaw also has high levels of potassium, calcium and iron, making it an excellent food source (Anon, 2010 ).

Presently, a variety of soft drinks are being produced in the country e.g. sweetened carbonated soft drinks, still beverages containing fruit juice and soda water. Among these, the share of fruit juice wine based beverages is presently quite small as compared to synthetic carbonated drinks. Gradually, there is a distinct shift towards fruit juice wine based beverages for obvious advantages of the higher nutritional, medicinal and calorie values over the synthetic aerated beverages (Svans, 2008). It has been reported that fruits and nuts form an integral part of the African diet and are consumed as relishes and snacks. Fruits are used in the production of beverages (Anon, 2007).

Wine has been made from almost every type of fruit, herbs, and flowers (Anon, 2007). Also documented is the chemical composition of edible parts of several fruits such as apple, apricots, peaches, berries, black currant cherries, plumbs, rosehips that are used in making wine (Anon, 2007). Therefore, in view of the above benefits and the perishability of the fruits; its conversion into a value added product like wine will be very useful.

## 2. Materials and Methods

### 2.1 Materials

The water melon (*Citrullus lanatus*) and pawpaw (*Carica papaya*) fruit used for this research work were bought from Igbona market Osogbo, Osun State, Nigeria. The equipment used include blender, weighing balance, electric stove, measuring cylinder, pH meter and hand-held refractometer (ATAGO-S-28E). Other materials used include cooking pot, washing bowls, stainless steel knife, water, napkin, stirrer, mechanical sieve, water melon (*Citrullus lanatus*), sugar, yeast, pawpaw (*Carica papaya*), packaging materials (bottles, cork, foil, wine) and preservatives (potassium metabisulphite).

### 2.2 Methods

The fruits were thoroughly sorted and graded to remove bad ones from the lot. The sorted fruits were washed to remove adhering soils, dirt and extraneous materials. The fruits here thereafter peeled, sliced and seeds removed. It was then diced, blended and sieved. Sugar and yeast were then added and left to ferment for 5-7 days. It was then clarified, preserved using potassium metabisulphite (KMS), pasteurized and then mixed together in various ratios, then labeled, bottled and sealed.

**Table 1.0: Sample Codes and Designation**

Samples	Pawpaw Juice	Watermelon Juice
389	90	10
421	80	20
561	70	30
672	60	40
784	50	50
Control	Table wine	

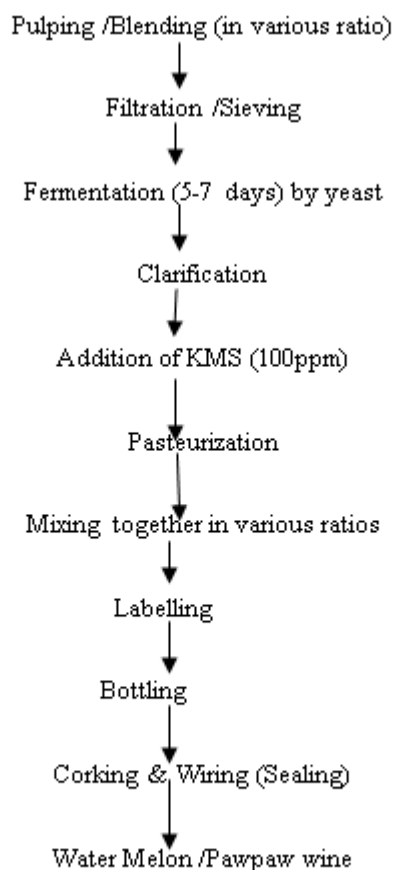


Figure 1: Flow Chart for the production of Pawpaw and Watermelon Wine

## 2.3 Physico – chemical Qualities

### 2.3.1 Titratable acidity determination

The wine was thoroughly mixed and filtered using muslin cloth. Five millilitres of filtrate was dissolved in previously boiled distilled water and made to 50 ml mark. 5 ml aliquot of the sample solution was taken and titrated with 0.1 N NaOH using phenolphthalein solution as indicator. Titratable acidity was calculated as percent citric acid (AOAC, 2005).

### 2.3.2 Total solids determination

Two millilitres (2 ml) of the wine was weighed into a dried and pre-weighed glass crucible. The crucible with its content was evaporated by putting it on a boiling water bath and dried to a constant weight in an oven at 70°C. The insoluble solids were calculated as a percentage of the sample (AOAC, 2005).

### 2.3.3 Total sugars determination

The concentration of soluble sugars was determined using a handheld Bellingham and Stanley refractometer (Bellingham and Stanley limited, 61 Markfield Road, London, England) at 20°C (AOAC, 2005).

### 2.3.4 Specific gravity determination

The specific gravity of the sample was determined using the picnometer-specific gravity bottle. The bottle was washed, rinsed and dried. The empty bottle was weighed and mass recorded as  $M_1$ . The bottle was emptied, rinsed, and filled with water and weighed, mass recorded as  $M_2$ . The specific gravity was calculated (AOAC, 2005).

### 2.3.5 P<sup>H</sup> measurement

The p<sup>H</sup> was measured using a P<sup>H</sup> meter, digital model EA513-055, ELE, England standardized with buffer solution of 4.0 and 7.0 (AOAC, 2005). The glass electrode of pH meter was dipped in 30mls of the beverage sample measured into a curvette at ambient temperature and was allowed to stabilize for sometimes after which the reading was taken.

### 2.3.6 Fat determination

This was carried out using the method of AOAC (2005). Clean and dried thimble was weighed (W<sub>1</sub>) and 5g oven dried sample was added and re-weighed (W<sub>2</sub>). Round bottom flask was filled with petroleum (ether 40-60)<sup>0</sup>C up to ¾ of the flask. Soxhlet extractor was fixed with a reflux condenser to adjust the heat sources so that the solvent boils gently, the samples were put inside the thimble and inserted into the soxhlet apparatus and extraction under reflux was carried out with petroleum ether for 6 hours. After the barrel of the extractor is empty, the condenser was removed and the thimble was removed, taken into the oven at 100<sup>0</sup>C for 1 hour and later cooled in the dessicator and weighed again (W<sub>3</sub>).

$$\% \text{ Fat} = \frac{\text{Weight loss of sample (extracted fat)} \times 100}{\text{Original weight of sample}} \text{-----} (1)$$

$$\% \text{ Fat} = \frac{W_2 - W_3 \times 100}{W_2 - W_1} \text{-----} (2)$$

### 2.3.7 Determination of crude protein

About 1g of the samples was weighed into micro Kjeldahl digestion flask and one tablet of Selenium catalyst was added. The mixture was digested on an electrothermal heater until clear solution was obtained. The flask was allowed to cool after which the solution was diluted with distilled water to 50ml and 5ml of this was transferred into the distillation apparatus, 5ml of 2% boric acid was pipetted into a 100ml conical flask (the receiver flask) and four drops of screened methyl red indicator were added. About 50% NaOH was continually added to the digested sample until the solution turned cloudy which indicated that the solution had become alkaline. Then distillation was carried out into the boric acid solution in the receiver flask with the delivery tube below the acid level. As the distillation was going on, the pink colour solution of the receiver flask turned blue indicating the presence of ammonia. Distillation was continued until the content of the flask was about 50ml after which the delivery of the condenser was rinsed with distilled water. The resulting solution in the conical flask was then titrated with 0.1M HCl (Pearson, 1976, (AOAC, 2005).

### Calculations:

$$\% \text{ Nitrogen} = \frac{\text{Titre value} \times 0.1 \text{HCl} \times 0.014 \times 100 \times 50}{\text{Original weight of the sample} \times 5} \text{-----} (3)$$

### 2.3.8 Determination of dry matter and moisture content.

About 2ml of each sample was measured into a previously weighed crucible, dried over water for sometimes. The crucible plus sample taken was transferred into the oven set at 100<sup>0</sup>c to dry to a content weight for 24hour overnight. At the end of 24hours, the crucible plus sample was removed from the oven and transfer to the desiccator, cooled for ten minutes and weighed (A.O.A.C, 2005). The weight of empty crucible plus sample was W<sub>1</sub> while the weight of crucible plus oven dried sample was W<sub>3</sub> (AOAC, 2005).

$$\% \text{ Dry matter} = \frac{W_3 - W_0 \times 100}{W_1 - W_0}$$

$$\% \text{ Moisture} = \frac{W_1 - W_3 \times 100}{W_1 - W_0}$$

$$\% \text{ Moisture content} = 100 - \% Dm$$

### 2.3.9 Vitamin C determination

Ascorbic acid (vitamin C) and vitamin B<sub>1</sub> content of the beverage were determined by the method of AOAC (2005).

**2.3.10 Vitamin A determination:** Reversed phase high performance liquid chromatography (HPLC) was used for the estimation of provitamin A content in the juice. 120 µl of homogenized juice was extracted with 500 µl of hexane. The mixture was vigorously shaken on an electronic shaker for 4 min, centrifuged for 2 min at 10,000rpm and the supernatant pooled. The extraction process was repeated. The pooled supernatant was evaporated to dryness under Nitrogen (N<sub>2</sub>) gas and redissolved in 120 µl mobile phase (1% tetrahydrofuran in methanol). The resulting aliquot (120 µl) was then injected into the HPLC (C-R6A Chromatopaa, Shimadzu Cooperation, Japan) column with ultraviolet detection (UV-VIS) spectrophotometric detector, Shimadzu, Japan) at 450 nm. A standard was prepared and chromatographed. Areas corresponding to the standard retention time were identified and used in the estimation of vitamin A content in the beverages samples.

### 2.4 Sensory Evaluation

Using multiple comparison tests, sensory evaluation of the pawpaw-watermelon wine blend samples was carried out by 9 trained panelists. In order to eliminate influence of flat taste, natural acidulant and sweetner were added to the drink after blending for uniformity of taste. Sensory attributes evaluated were taste, aroma, colour, consistency and overall acceptability using seven point hedonic scale of 1 to 7 where 1 indicates extremely like and 7 indicate extremely dislike (Larmond, 1979).

### 2.6 Microbiological Analysis

**2.6.1 Total viable count of bacteria (TVC):** The microbiological analysis was carried out according to Harrigan (1998). Plate count agar was used for enumeration of bacteria. A well homogenized sample was serially diluted with 0.1% peptone water up to 10<sup>-6</sup>. One ml aliquot from a suitable dilution was transferred aseptically into sterile petri dishes. To each plate about 15ml of melted and cooled PDA (Potato Dextrose Agar) was added. The inocula was evenly mixed with media by rotating the plates and allowed to solidify. The inverted plate was incubated for 48hours. The TVC (cfu/ml) was determined using a colony counter.

**2.6.2 Total coliform bacteria:** Mac Conkey broth was used for the detection of coliform bacteria by the multiple tube technique. The medium was distributed in 9ml quantities standard test tubes with inverted Durham tube and was then autoclaved for 20mins at 121°C. Well homogenized samples was serially diluted (10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup> and 10<sup>-4</sup>) with 0.1% peptone water. 1 ml from each dilution was aseptically inoculated into triplicate of 9 ml sterile Mac Conkey broth in standard test tube and incubated for 48hrs at 37°C. Positive tests gave gas in the Durham tubes and changed the color of the medium (Harrigan, 1998).

**2.6.3 Yeast and Mould Enumeration:** Potato dextrose agar (PDA) was used for enumeration of yeast and mould. Well homogenised samples were serially diluted with 0.1% peptone water up to 10<sup>-6</sup>. Aliquots (0.1ml) from a suitable dilution were transferred aseptically into solidified PDA plates. Samples were spread all over the surface of the plates using sterile bent glass rod. The plates were then incubated for 48 to 72hrs at 28°C. Counting (cfu/ml) was carried out by using colony counter (Harrigan, 1998).

### 2.7 Statistical Analysis

One way analysis of variance (ANOVA) using repeated measures was conducted on physico-chemical qualities and mean separation was done with Duncan's Multiple Range Test (DMRT) when significant (p<0.05) difference were observed, means were separated using Turkey's test (Snedecor, 1956) and descriptive statistics was done on the sensory attributes.

### 3.0 Results and Discussion

#### 3.1 Results

**Table 2.0: The Physicochemical Properties of the Formulated Wine**

Sample	D.M. (%)	M.C. (%)	A.C. (%)	C.P. (%)	T.S. (%)	S.G. (%)	B.L.	TTA	pH	VIT C (mg/100g)	VITB <sub>1</sub> (mg/100g)	VIT A (mg/100g)
561	5.40	94.60	0.05	1.14	2.00	0.784	12.00	1.00	3.00	9.00	10.00	24.00
389	5.37	94.63	0.07	1.13	1.99	0.788	12.01	1.15	2.80	8.85	9.96	23.70
421	5.35	94.65	0.09	1.12	1.98	0.792	12.02	1.23	2.60	8.70	9.92	22.80
910	5.32	94.68	1.02	1.11	1.97	0.796	12.03	1.31	2.40	8.55	9.88	21.67
672	5.30	94.71	1.05	1.10	1.96	0.800	12.04	1.42	2.20	8.40	9.84	20.54
Control	5.01	94.99	1.00	1.05	1.50	1.020	13.00	1.70	2.00	7.00	8.80	20.00

**Key:** D.M. rep dry matter, M.C. rep moisture content, A.C. rep Ash content, C.P. rep crude protein, T.S. rep total solid, S.G. rep Specific gravity, B.L. rep Brix Level, TTA rep Titratable Acidity and VIT. rep Vitamin

**Table 3.0: The Sensory Result of the Formulated Wine**

Sample code	Colour	Flavour	Taste	Mouth feel	Overall Acceptability
389	63	53	59	64	66
421	65	56	57	62	67
561	67	59	53	60	69
672	69	62	60	67	72
784	61	54	54	55	70
Control	60	60	50	52	65

**Table 4.0: The Microbial Load of the Formulated Wine (cfu/ml)**

Sample	TVC	TCC	TYC	TMC
389	$1.03 \times 10^{-2}$	Nil	Nil	Nil
421	$1.02 \times 10^{-2}$	Nil	Nil	Nil
561	$1.02 \times 10^{-2}$	Nil	Nil	Nil
672	$1.01 \times 10^{-2}$	Nil	Nil	Nil
784	$1.01 \times 10^{-2}$	Nil	Nil	Nil
Control	$1.00 \times 10^{-2}$	Nil	Nil	Nil

**Key:** TVC rep Total viable count, TCC rep Total coliform count, TYC rep Total yeast count, TMC rep Total yeast count

## 3.2 Discussion

### 3.2.1 The Physicochemical Properties of the Formulated Wine

The result of the physicochemical analysis conducted is shown in Table 2.0. Total titratable acidity of the wine sample ranged from 1.00 to 1.70% citric acid equivalent of the wine. This is similar to the commercial recommendation of acidity for wine (Srivastava and Kumar, 1998). There exists a correlation between  $P^H$  and acidity of the sample. The higher the acidity, the lower the  $P^H$  of the wine. A similar study conducted by Abbo *et al.*, (2006) revealed that there is a corresponding reduction in pH as the acidity increased in sour sop juice. The pH of the wine was below 4.00. Thus was supported by Cole, *et al.*, (2000). The total soluble solid was between 12.00 to 13.00° brix while  $P^H$  ranged from 2.00 to 3.00. The pH is slightly acidic, this confers stability on the wine sample (Ihekoronye and Ngoddy 1985) and the specific gravity ranged from 0.784 to 1.020. This was supported by Nidhi *et al.*, (2008)

The formulated wine provides a considerable amount of vitamin C. About 27.08 mg/ 100g has been reported to provide more than one third of the daily requirements of vitamin C (Hou *et al.*, 2001). However, vitamin C contents of the wine samples were moderate (7.00 – 9.00 mg/100 g). The reduction in vitamin C content may be due to the effect of heat during pasteurization. The ascorbic also acts as an antioxidant to help prevent molecular changes caused by oxidation and as a promoter of iron absorption (Wardlaw, 1999).

Vitamin A and B<sub>1</sub> content of the pawpaw-watermelon wine ranged from 20.00 to 24.00 mg/100g and 8.80-10.00mg/100g respectively. The vitamin A and B<sub>1</sub> content in the beverage is considerably higher than in most commercial drinks as reported by Collins, 1981. Vitamin A and B<sub>1</sub> deficiency is one of the major public health problems in developing countries, Nigeria inclusive. Thus, beverage will be more welcomed by consumers who are now more conscious about the nutritional content of what they consume.

The moisture content of the non-alcoholic wine ranged between (94.60--94.99) %. High moisture content makes beverage suitable as a refreshing and quench-thirsting product which is characteristic of good beverage. This is similar to the report given by Oshuntogun and Aboaba, (2004). This is comparably higher than the moisture content of zobo drink (88.88)%, sorghum stem sheath beverage (88.95) % and that of kunnu 84.90% (Agarry *et al.*, 2010; Egbera *et al.*, 2007).

The protein content of the beverage ranged between 0.05-1.00%. The low protein content may be attributed also to the effect of heat process involved in its extraction which might have destroyed some amino acids with consequent reduction in total nitrogen content of the resulting beverage (Oluwaniyi *et al.*, 2009). However, the protein contents of the formulated wine (1.70-1.90)% was found to be higher than that of the sobo drink (0.046) % (Agarry *et al.*, 2010).

The watermelon-pawpaw wine has low fat content which ranged between (0.015-0.030) percent. This might be attributed to the effect of direct heat on fat soluble components of the beverage during the process of extraction (Adelakun *et al.*, 2009). The fat content of the non- alcoholic beverage is lower than that of sobo drink (0.15) % (Egbera *et al.*, 2007).

### 3.2.2 Organoleptic attributes of the formulated wine

The wine samples were subjected to sensory evaluation and the scores were subjected to ANOVA. The result is shown in Table 3.0, the sample coded 672 was significantly different from all other samples in terms of colour, taste, flavor, aroma, consistency and general acceptability at 5% level of significance according to General Linear models (GLM). The beverage had a high rating sensory score in consumer test, thus can be prepared for commercial purpose to serve as a special beverage with similar constituents as other already existing commercial beverages.

### 3.2.3 Microbial analysis of the formulated wine

The result of microbial analysis has shown in Table 4.0 revealed that the quality of the wine beverage. The microbial analysis of the pawpaw-watermelon wine revealed that there was no mould, yeast and coliform growth while the total viable count ranged between 1.00-1.03 x 10<sup>-2</sup>. This implies that the beverage was produced under hygienic conditions and is safe for human consumption (Frazier and Westhoff, 1988). Therefore,



the total viable count was within the acceptable limit according to Sri Lanka standard for all drinks / beverages and juices (Ghana Standard Board, 1995). The heat treatment was sufficient to destroy microbial load in the wine beverage. Carter *et. al.*, 2007 reported that many products that could safely be maintained sterile by a pasteurization process alone could be doubly preserved by the addition of potassium metabisulphates. The sulphites inhibit yeast, moulds and bacteria (Doughari and Elmahmood, 2007). The shelf life evaluation prevailed that the wine sample could be stored for six months at room temperature of  $30 \pm 2^{\circ}\text{C}$  without any significant changes in the quality characteristic. According to the microbial studies during shelf life evaluation microbial colonies were not observed in the sample. Therefore, the formulated wine is safe for consumption for a minimum period of six months.

#### 4. Conclusion and Recommendation

This research work was designed to reduce seasonal wastage of the fruit thereby increasing their shelf life through the process of development of juices and beverages.

The physicochemical analysis revealed that pawpaw-watermelon wine is nutritionally rich in protein, fat and vitamin C, A and B<sub>1</sub>. The organoleptic analysis showed that sample code 672; 60% PJ: 40% WMJ was selected as the most preferred treatment based on organoleptic point of view. According to the microbial test, tolerable numbers of bacterial colonies were found in the products. Therefore, the quality of the pawpaw-watermelon juice is within the acceptable quality range specified by Ghana Standards Board (1995) for juices and beverages. Therefore, it is safe for human consumption.

Based on the above conclusion, pawpaw watermelon wine could be regarded as after meal drink, ready to serve, refreshing drink with higher nutritional, medicinal and calorie value for the populace over synthetic aerated beverages which are harmful to human health.

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